УДК: 577.3

Effect of Combined Magnetic Fields with Very Weak Alternating Component on Duration of Pupal Metamorphosis Stage in Mealworm Beetle *Tenebrio molitor*

Vadim V. Novikov[1], **Elena V. Yablokova**[1], **Nadezhda I. Novikova** [2]

[1]Institute of Cell Biophysics, Russian Academy of Sciences,
Institutskaya St., 3, Pushchino, Moscow Region, 142290, Russia
e-mail: docmag@mail.ru
[2]Branch of Shemyakin and Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Sciences,
6 avenue of Science, Pushchino, Moscow Region, 142290, Russia

Abstract

It is shown that an exposure of pupae of the mealworm beetle *Tenebrio molitor* to the combined static (42 μT) and very weak extremely low-frequency alternating collinear magnetic fields exerts different influence, depending on the frequency of the alternating magnetic field (AMF), on duration of metamorphosis processes in these insects. For instance, a no less than 8 hour-exposure of young pupae to weak combined magnetic fields, adjusted to the frequency of ion cyclotron resonance for Ca^{2+} (32.2 Hz) at the AMF of 100 and 250 nT, inhibits metamorphosis process – a transitional stage from pupae to imago lasts longer. In contrast, a stimulating effect was observed when adjusted to cyclotron frequency for ion of glutaminic acid (4.4 Hz) at the amplitudes of 40, 100 and 250 nT. At the weaker amplitudes of the AMF this effect is not seen. For instance, an exposure at a frequency of 4.4 Hz, the amplitude of AMF 6, 16 nT and at a frequency of 32.2 Hz, the amplitude of AMF 6, 16 and 40 nT exerts no noticeable effect on the duration of the pupal metamorphosis stage.

Key words: weak magnetic fields, metamorphosis, mealworm beetle

Новиков В. В., Яблокова Е. В., Новикова Н. И., Влияние комбинированных магнитных полей с очень слабой переменной компонентой на продолжительность стадии куколки метаморфоза у мучного хрущака *Tenebrio molitor* // «Живые и биокосные системы». – 2017. – № 20; URL: http://www.jbks.ru/archive/issue-20/article-6

Влияние комбинированных магнитных полей с очень слабой переменной компонентой на продолжительность стадии куколки метаморфоза у мучного хрущака *Tenebrio molitor*

Новиков Вадим Викторович [1], Яблокова Елена Владиславовна [1], Новикова Надежда Ивановна. [2]
[1]ФГБУН Институт биофизики клетки РАН
ул. Институтская, 3, г. Пущино Московской области, 142290, РФ
e-mail: docmag@mail.ru
[2]Филиал ФГБУН Института биоорганической химии им. М.М. Шемякина и Ю.А. Овчинникова РАН
проспект Науки, 6, г. Пущино Московской области, 142290, РФ

Аннотация

Показано, что воздействие комбинированными постоянным (42 мкТл) и коллинеарным ему очень слабым сверхнизкочастотными переменными магнитными полями на куколок мучного хрущака *Tenebrio molitor* оказывает в зависимости от частоты переменного поля разнонаправленное влияние на продолжительность процессов метаморфоза у этих насекомых. Так, не менее чем 8 часовая экспозиция молодых куколок в слабых комбинированных магнитных полях, настроенных на частоту ион-циклотронного резонанса для иона Ca^{2+} (32,2 Гц) при амплитудах переменного поля 100 и 250 нТл, тормозит метаморфоз – время превращения куколок в имаго увеличивается. При настройке на циклотронную частоту для иона глутаминовой кислоты (4,4 Гц) при амплитудах 40; 100; 250 нТл отмечен противоположный стимулирующий эффект. При некоторых более слабых амплитудах переменного поля эффект отсутствует. Например, воздействие на 4,4 Гц при 6; 16 нТл и на 32,2 Гц при 6; 16; 40 нТл не оказывает заметного влияния на изучаемый параметр.

Ключевые слова: слабые магнитные поля; метаморфоз; мучной хрущак

Introduction

A concept of ion cyclotron resonance (ICR) in biological systems in weak combined static and alternating collinear magnetic fields suggested by A.R. Liboff [10] proved to be perspective [2,14,22] In particular, our experiments have shown for instance that, ion-current changes were observed at ICR frequencies of amino acids in cell-free systems at the intensities of alternating components of ~ 40 nT [14,23]. Further in our works with different biological objects: planarians [15], tumor-bearing animals [16], mouse models of Alzheimer disease [3] we found different effects of the biological action of weak combined magnetic fields (CMF) with a very weak alternating component (tens and hundreds of nT) with a response at frequencies corresponding to ICR conditions for a number of organic and inorganic ions.

Of interest is exploring the effect of magnetic fields mentioned on metamorphosis processes. The choice of the model for study was made based on the previous reports on the sensitivity of metamorphosis processes in the mealworm beetle *Tenebrio molitor* towards the effect of weak CMF [4] and extremely high frequency emissions [19]. In Ermakov and Lednev [4], a stimulating effect was revealed in the weak CMF adjusted to parametric resonance for Ca^{2+} and K^{+} at a ratio of the alternating component to static one 1.8 : 1 and also in the very weak alternating magnetic field (AMF) at the amplitude of 1,6 μT and frequency of 76 Hz adjusted by V.V. Lednev hypothesis to the magnetic moment of hydrogen atoms [8]. In all these cases the authors report the shortening of pupal metamorphosis stage in the mealworm beetle *Tenebrio molitor* [4]. We then made an experiment [17] and observed the sensitivity of metamorphosis stage in the mealworm beetle *Tenebrio molitor* exposed even to the weaker alternating component of CMF (250 nT). It was shown that the effect of magnetic field depends on the frequency of the alternating component. For instance, in static magnetic field (SMF), 42 μT, at a frequency of the alternating component 4.4 Hz (corresponding to cyclotron resonance frequency for ion form of glutamic acid) morphogenesis is stimulated – the duration from pupal stage to imago was shorter. At cyclotron resonance frequency for Ca^{2+} of 32,2 Hz, the opposite effect was recorded. An exposure at cyclotron resonance frequency for K^{+} of 16,5 Hz resulted in no noticeable effect on the duration of this stage. We decided to continue our study in a range of the weakest intensities of the AMF. For this purpose in our further experiments the biological objects were put in a special chamber shielded against the external magnetic field to reduce external geomagnetic pulsations and technogenous disturbances.

Material and methods

Object of study

Adult individuals of the mealworm beetle *Tenebrio molitor (Colepptera, Tenebrionidae)* were kept in crystallizers under darkened conditions with bran at a constant temperature of 24-25°C. Every week the beetles were transferred to fresh bran. The beetle eggs-containing bran was kept until emergence of larvae. Partly grown larvae were transplanted into new bran. The pupae of beetles that emerged from larvae were collected once a week and placed into a separate crystallizer with bran. At a next stage the imago insects emerged from the pupae. A new generation of insects was then produced. This stage-by-stage rearing of the culture made it possible to select synchronized developing insects.

Experimental procedures

Young pupae were collected at the start of the pupal stage, their skin was whitish in color and pupal development lasted about four hours. The pupae collected were divided into experimental and control groups 50 pupae in each group. Pupae in glass beakers with bran were put at one and the same time in two identical chambers shielded from external magnetic fields. In one chamber SMF (42 ± 0.1 μT) and AMF were generated (experiment). Only SMF (42 ± 0.1 μT) was produced in the second chamber (control). Additional groups of pupae were exposed to local geomagnetic field with induction of 42 ± 0.1 μT μT and technogenous background of up to ~ 30 nT, 50 Hz (extra control).

Pupae were exposed in darkened conditions at temperature of 24-25º C. Each series of the experiments was repeated no less than three times.

Pupae were observed until the imago insects emerge two times a day – in the morning (9 a.m.) and in the evening (6 p.m.). The number of the emerged beetles in groups was determined and the time period from pupae to beetle eclosion was compared in experimental and control groups.

Characterization of magnetic fields and magnetic field forming technique

Two identical chambers were used in the experiments. These chambers consist of external magnetic shield made of permalloy, as a cubic shaped box, with shielding efficiency of ~ 650, two located inside the shield coaxially arranged rectangular magnetic coils that generate AMF and SMF when connected to a remote sine-wave generator and constant-current source. Inhomogeneity of fields in the area of the experiment was not greater than 1%. The magnitudes of acting magnetic fields were determined by measurement using a ferroprobe magnetometer Mag-03 MS (Bartington, Oxford, UK).

A series of the experiments was made at two frequencies of 4.4 and 32.2 Hz at different amplitudes of 6, 16, 40, 100, 250 nT.

Pupae from the experimental group were exposed to CMF for the first 4, 8, 12 and 24 hours from the start of the pupal stage, then the alternating component of the magnetic field was powered down, only SMF (42 ± 0.1 μT) was produced.

Statistical analysis. Statistical analysis was performed using the Student *t*-test. The value $P < 0.05$ was considered to be statistically significant.

Results

As it is shown in Fig. 1 an 8-hour exposure of young pupae selected at the very start of the pupal stage from the chamber shielded against the external magnetic field to CMF at a ICR frequency for Ca^{2+} of 32.2 Hz at the amplitude of 250 nT and SMF of 42 μT resulted in a noticeably elongated period of the pupal stage. Beetle eclosion from pupae in the experimental group began 9.2 hours later compared to those in the control one (Fig. 2) but a duration of beetle eclosion remained the same. A shortening of the time period of an exposure up to four hours resulted in no effect of these CMF (Fig. 2). The level of the effect of the prolonged exposure up to 12 hours and then up to 24 hours was not higher than that observed under the 8-hour exposure (Fig. 1). Taking into account that weaker amplitude of AMF was exploited in our subsequent experiments, we chose a 8-hour exposure to the magnetic field. A diminution in the amplitude of the AMF at the frequency of 32.2 Hz from 250 nT down to 6 nT with 2.5 step led to the same inhibiting effect observed at 100 nT (Fig. 3). At the amplitude of 40, 16 and 6 nT no noticeable effect of magnetic field was registered.

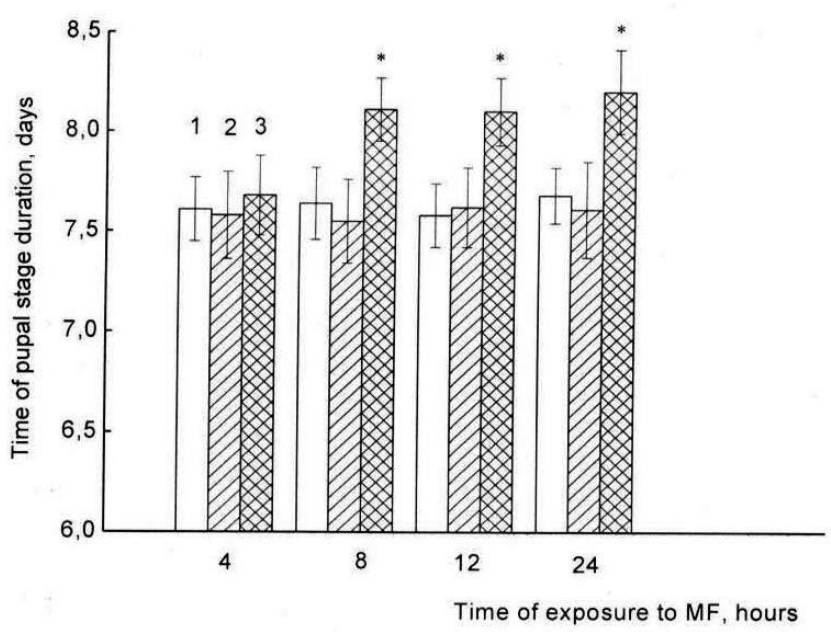

Fig. 1. The dependence of the time of the pupal stage duration (mealworm beetles Tenebrio molitor) on combined MF exposure time at a frequency of 32.,2 Hz (amplitude, 250 nT), static field 42 μT.

1. *– control 1 (chamber) DC MF 42 μT*
2. *- control 2 (geomagnetic field) DC MF 42 μT*
3. *– experiment*

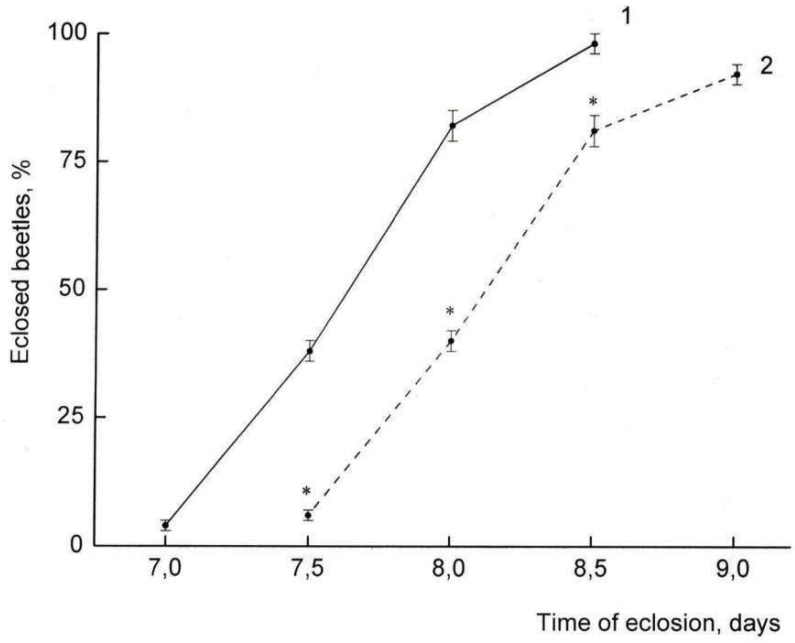

Fig. 2. Dynamics of eclosion of beetles from the pupa in condition of an 8-hour exposure to combined magnetic field at a frequency of 32.2 Hz (amplitude 250 nT), static magnetic field 42 μT.

1 – control 1 (chamber) DC MF 42 μT

2 - control 2 (geomagnetic field) DC MF 42 μT

3 – *experiment*

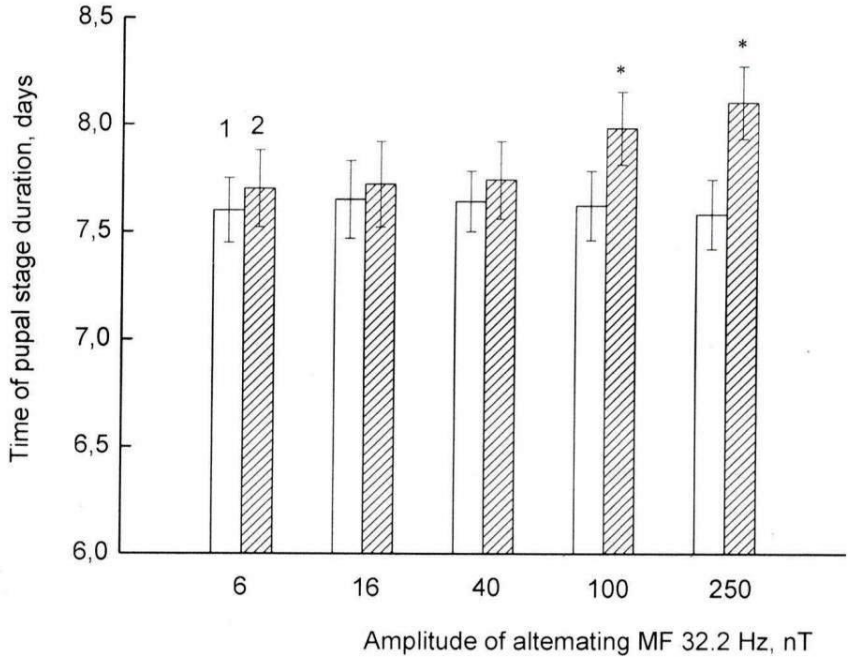

Fig. 3. The dependence of the time of the pupal stage duration (mealworm beetles *Tenebrio molitor*) in condition of an 8-hour exposure to combined magnetic field on the amplitude of the alternating magnetic field at a frequency of 32.2 Hz, static magnetic field 42 μT.

1 – control (chamber) DC MF 42 μT

2 - experiment

It should be noted that no statistically significant differences between the control group in SMF of 42 μT in the shielded chamber and the control group in natural geomagnetic field at the same magnitude of the static field were found (Fig. 1).

The change of the frequency of the alternating component of the CMF from 32.2 Hz to 4.4 Hz (corresponding to ICR frequency for ion form of glutamic acid) at 250 nT in course of 8 hour exposure to CMF resulted in the inversion of the sign of the effect (the stimulating effect). The duration of the pupal stage shortened (Fig. 4). A decrease in the amplitude of the alternating component of the CMF up to 40 nT at the frequency of 4.4 Hz led to the same stimulating effect. At the amplitude of 16 and 6 nT no effect of magnetic field was registered (Fig. 4).

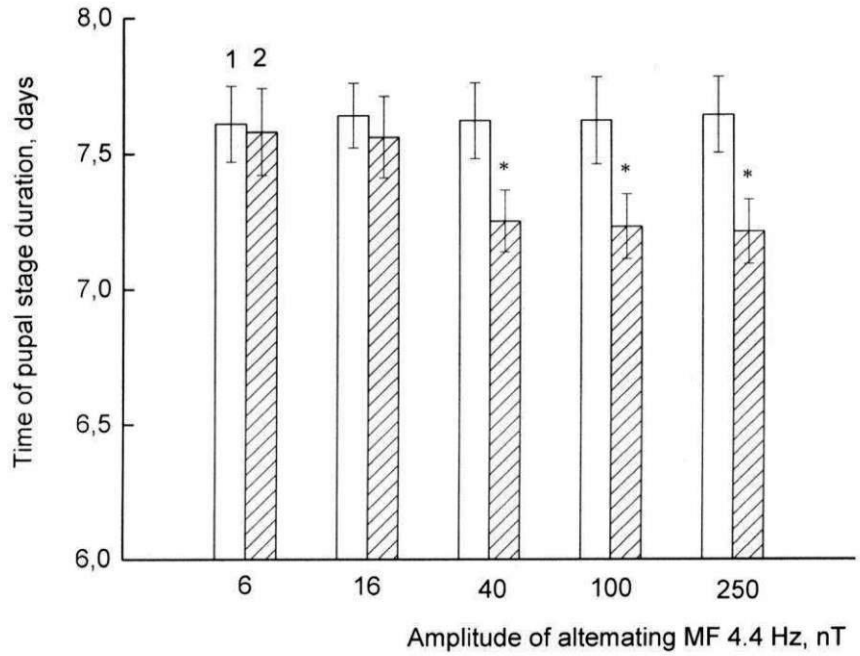

Fig. 4. The dependence of the time of the pupal stage duration (mealworm beetles Tenebrio molitor) in condition of an 8-hour exposure to combined magnetic field on the amplitude of the alternating magnetic field at a frequency of 4.4 Hz, static magnetic field 42 μT.

1 – control (chamber) DC MF 42 μT

2 - experiment

Discussion

The experiments focused on observation of the effect of the CMF with a very weak alternating component using at one and the same time two identical chambers (control and experiment) shielded against the external magnetic field with a built-in system of generation of magnetic fields made it virtually possible to exclude concurrent magnetic disturbances and increase a reliability of the results obtained. It should be noted that the interest in exploration of biological action of these very weak fields is associated with clearing up a biological role for natural magnetic pulsations of Pc 1 type with the frequencies from 0.2 to 5.0 Hz and amplitudes in a range from small doses to tens of nT that correlate, according to the findings of some epidemiological studies, for instance, with the incidence rate of acute disorders of blood circulation - strokes, heart attacks [7]. To determine a biological efficiency of the parameters comparable to the magnitude and frequency characteristics of the fields it appears to be reasonable to use a system of shielding against technogenic and natural magnetic fluctuations to compensate external magnetic fields present in this magnetobiology experiment.

On the whole, the data obtained point to the possibility of modulation (stimulation, inhibition) of the duration of metamorphosis process in the mealworm beetle *Tenebrio molitor* at the pupal stage with weak CMF adjusted to ICR frequencies of different biologically active ions (in our case ions of Ca^{2+} and ion forms of glutaminic acid). The adjustment of the frequency of CMF is therefore a crucial parameter for a biological response. So, at ICR frequency of Ca^{2+} the metamorphosis process ran for a longer period of time, at the frequency of ion form of glutaminic acid the duration of this process was shorter. These findings are in agreement with the effects relating to ICR [11].

The results obtained provide an opportunity to talk about a threshold magnitude of the AMF component for a biological response. As to the two studied frequencies 4.4 Hz and 32.2 Hz with collinear SMF 42 μT (geomagnetic range of the intensity) an object response was recorded at the amplitude of the AMF no lower than 40 nT (at 32.2 Hz it was observed at 100 nT). At these two frequencies one-step diminution in the AMF from 40 nT down to 6 nT led to no biological response. Even given the response of the biosystem to the action of the weak magnetic field is often of nonlinear nature, there exist “gaps” in biological activity or multipeak dependence [6,13].

The results obtained with the model (duration of metamorphosis processes in pupae of *Tenebrio molitor*) used in our experiments are significant and point to the possible existence of a threshold in a range of ~ 40 nT in a very low-frequency range. Taking into account our experimental material it appears that the biologically active magnitude of the weak alternating magnetic field depends on the properties and parameters of the experimental model, namely on the concentration of the field receptors or particular features of their genome systems.

The effect of very weak AMF and SMF on morphogenetic processes (division, regeneration) has been considerably studied in more detail using other invertebrates – planarians [15,1,21]. In our experiments with planarians we also registered the biological response to the effect of magnetic field at the amplitude of 40 nT [15].

It should be noted that the magnitudes of the AMF component which were determined in our experiments are comparable to the value of daily variations in geomagnetic field [12]. Although the frequency range of the extremely low frequency magnetic fields used in our experiment and daily variations in the constant component of the geomagnetic field are different, the magnitudes of these parameters are close. This is one of the confirmations of a possible general biological role of these variations.

While analyzing molecular and cellular mechanisms of the effect of very weak magnetic field on the metamorphosis of the mealworm beetle *Tenebrio molitor,* it should be noted that histoblasts of imaginal disks in insects are an analog to neoblasts (stem cells) in the planarians that change their proliferative activity being exposed to a weak magnetic field [20,22]. The impact of very weak magnetic field

on insect signal transduction systems is not excluded either. It should be also taken into consideration that weak magnetic fields may influence the hormonal control of metamorphosis in the developing insects. In this regard it is reasonable to highlight that the effect of SMF, 320 mT on the morphology of neurosecretory cells of corpus albatum in pupa *Tenebrio molitor* [18] and the effect of the alternating low frequency magnetic field, 2 mT on the hormonal status in caterpillar *Lymantria dispar* [5] have been shown.

The model system explored in this study – metamorphosis of the mealworm beetle *Tenebrio molitor* – can be most likely useful in research of teratogenic effects of weak magnetic field the occurrence of which can be expected during beetle eclosion. This usage is reported in [4], an exposure to CMF adjusted to parameteric resonance Ca^{2+} at SMF of 40,5 μT and the AMF frequency of 31 Hz and amplitude of 74,5 μT caused abnormal development in the increased amount of insects.

In conclusion it should be noted that recorded different biological effects (stimulation, inhibition), a sign and the intensity level of which depend on the frequency and the magnitude of magnetic field, point to the possibility of regulating a state of the bioobject applying the CMF with a very weak alternating component.

References

1. Belova, N.A., Ermakov, A.M., Znobishcheva, A.V. et al. (2010). The influence of extremely weak alternating magnetic fields on the regeneration of planarians and the gravitropic response of plants. Biophysics 55:623-627.
2. Blackman CF, Blanchard JP, Benane SG, House DE, Elder JA. 1998. A double blind test of magnetic field effects on neurite outgrowth. Bioelectromagnetics 19: 204-209.
3. Bobkova, N.V., Novikov, V.V., Medvinskaya N.I. et al. (2010). The weak combined magnetic fields induce the reduction of brain amyloid-β level in two animal models of Alzheimer's disease. Eur J Oncol Library 5:235-246.
4. Ermakov, A.M., Lednev, V.V. (2010). Effect of weak combined magnetic fields on the metamorphosis of the mealworm beetle tenebrio molitor. Biophysics, 55:633–636.
5. Ilijin, L., Vlahovic, M., Mrdakovic, M. et al. (2011). The effects of acute exposure to magnetic fields on morphometric characteristics of bombyxin-producing neurosecretory neurons in gypsy moth caterpillars. Int J Radiat Biol 87:461-71.
6. Ishido, M., Kabuto, M. (2001). Magnetic fields (MF) of 50 Hz at 1.2 microT as well as 100 microT cause uncoupling of inhibitory pathways of adenylyl cyclase mediated by melatonin 1a receptor in MF-sensitive MCF-7 cells. Carciogenesis 22: 1043–1048.
7. Kleimenova, N.G., Kozyreva, O.V., Breus, T.K., Rapoport, S. I. (2007) Seasonal variations in myocardial infarctions and the possible biotropic influence of short-period geomagnetic pulsations on the human cardiovascular system. Biophysics 52: 625–631.
8. Lednev, V. V. (2003). Biological effects of the extremely weak alternating magnetic fields: the identification of primary targets. In Sidorin A., ed. Modeling of geophysical processes (pp. 130–136). O.Yu. Shmidt United Institute of the Earth's Physics RAS.
9. Lednev VV, Belova NA, Rojdestvenskaya ZYe, Tiras KP. 2003. Biological effects of weak alternating magnetic fields and biological precursors of earthquakes. In Sidorin A., ed. Modeling of geophysical processes (pp. 3–11). O.Yu. Shmidt United Institute of the Earth's Physics RAS (in Russian).
10. Liboff, A. R. (1985) Geomagnetic cyclotron resonance in membrane transport. J Biol Phys 13:99-102.
11. Liboff, A.R. (2010a) A role for the geomagnetic field in cell regulation. Electrom Biol Med 29:105-112.
12. Liboff A.R. (2010b) Weak low-frequency electromagnetic fields are biologically interactive. Eur J Oncol Library 5:51-62.

13. Liburdy, R.P., Sloma, T.R., Sokolic, R. (1993) EMF magnetic fields, breast cancer, and melatonin: 60 Hz field block melatonin's oncostatic action of ERþ breast cancer cell proliferation. J Pineal Res 14:89 –97.
14. Novikov, V.V., Zhadin, M.N. (1994) Combined action of weak static and alternating low-frequency magnetic fields on ionic current in aqueous amino acid solutions. Biophysics 39:41 – 45.
15. Novikov, V.V., Sheiman, I.M., Fesenko, E.E. (2008) Effect of weak static and low-frequency alternating magnetic fields on the fission and regeneration of the planarian *Dugesia (Girardia) tigrina.* Bioelectromagnetics 29: 387-393.
16. Novikov, V.V., Novikov, G.V., Fesenko E.E. (2009) Effect of weak combined static and extremely low-frequency alternating magnetic fields on tumor growth in mice bearing the Ehrlich ascites carcinoma // Bioelectromagnetics 30:343-351.
17. Novikov, V.V., Sheiman, I.M., Yablokova, E.V., Fesenko, E.E. (2014) Modulating effect of weak combined magnetic fields on the duration of metamorphosis mealworms *Tenebrio molitor*. Biofizika 59:1157-1160, (in Russian).
18. Peric-Mataruga, V., Prolic, Z., Nenadovic, V. et al. (2008) The effect of a static magnetic field on the morphometric characteristics of neurosecretory neurons and corpora allata in the pupae of yellow mealworm Tenebrio molitor (Tenebrionidae). Int J Radiat Biol 84:91 – 98.
19. Sheiman, I. M.**,** Shkutin, M.F. (2003) Effect of weak electromagnetic radiation on larval development and metamorphosis of the yellow mealworm beetle Tenebrio molitor. Biophysics 48:99-104.
20. Sustar, A.**,** Schubiger, G. (2005) A transient cell cycle shift in Drosophila imaginal disc cells precedes multipotency. Cell 120:383-393.
21. Tessaro, L.W.E., Persinger, M.A. (2013) Optimal durations of single exposures to a frequency-modulated magnetic field immediately after bisection in planarian predict final growth values. Bioelectromagnetics 34:613-617.
22. Tiras, K.P., Srebnitskaya, L.K., Il'jasova, A.A., Lednev, V.V. (1996) The influence of weak combined magnetic fields on the rate of regeneration in planarian Dugesia tigrina. Biophysics 42:826 – 831.
23. Zhadin, M.N., Novikov, V.V., Barnes, F.S., Pergola, N.F. (1998) Combined action of static and alternating magnetic fields on ionic current in aqueous glutamic acid solution. Bioelectromagnetics 19:41– 45.